Remarks

Claims 107, 139 and 174 are amended herein. Claims 1-106, 115, 137, 141, 146, 148-156 and 168-171 were previously cancelled. Upon entry of the amendments set out herein claims 106-114, 116-136, 138-140, 142-145, 147, 157-167 and 172-174 will be pending.

Claim 107 is amended herein to recite the limitation "wherein the double stranded RNA sequences mediate inhibition of target gene expression." Support for this amendment can be found throughout the specification and particularly on page 8, paragraph 2, lines 14-17, and page 20, paragraph 3, lines 17-24.

Support for the amendment language of claims 139 and 174 is found, for example, in claim 109 and, for example, in the specification at page 8, lines 24-26.

Double Patenting:

Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting over claims 119-135 of copending Application No. 10/836,856.

Without conceding with regard to the appropriateness of the rejection, Applicants submit that whereas this is a provisional rejection, Applicants will consider filing a terminal disclaimer in the appropriate application upon notice that the claims are otherwise allowable.

Rejections under 35 U.S.C. § 112

Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are rejected under 35 U.S.C. § 112, for the introduction of new matter.

1. The Office Action recites the following (page 4, paragraph 3, lines 1-2 and page 4 paragraph 4, lines 1-3):

> "The limitation "wherein said two or more different double stranded RNA sequences are separated by cleavage sequences" is new matter...

> The discussion of such sequences for cleavage is disclosed in a general manner and there is no explicit support for **specifically where such cleavage sequences are placed in relation to the dsRNAs.** "

Applicants respectfully disagree for the following reasons and submit that there is sufficient guidance in the specification for the location of a cleavage site as presently claimed.

The specification recites (page 8, paragraph 2, lines 14-17):

> "The polynucleotide sequences described herein may employ a **multitarget or polyepitope approach**, e.g., encoding sequences of **more than one gene** of a single target pathogen **or against more than one target** pathogen, or other category of target desired to be silenced."

The specification also recites the following (page 20, paragraph 3, lines 17-24):

> "The vectors designed to produce dsRNAs of the invention may desirably be designed to generate two or more, including a number of different dsRNAs homologous and complementary to a target sequence. This approach is desirable in that a single vector may produce many **independently operative dsRNAs** rather than a single dsRNA molecule from a single transcription unit and by producing a multiplicity of different dsRNAs, it is possible to self-select for optimum effectiveness. Various means may be employed to achieve this, including autocatalytic sequences as well as **sequences for cleavage to create random and/or predetermined splice sites**."

The specification describes a "multitarget" approach to include dsRNAs directed at more than one gene or target. The specification further describes the production of "independently operative dsRNAs" and provides guidance for the use of cleavage sequences to achieve the production of such multiple dsRNAs. It is easily understood from the description of a "multitarget approach," in combination with the reference to "independently operative dsRNAs," that the invention as presently claimed permits the generation of at least two distinct dsRNA molecules. The separation of such molecules from the claimed multitarget partially double-stranded RNA molecule can be achieved, as described in the specification, by the inclusion of "sequences for cleavage." Thus, one of skill in the art would understand, based on the teachings of the specification, that in a multitarget approach to produce "independently operative dsRNAs" from a

multitarget partially double stranded RNA molecule one must put a cleavage sequence **between** the "two or more dsRNA sequences."

Therefore, Applicants submit that one of ordinary skill in the art would understand based on the present specification that in a multitarget approach, two or more different double stranded RNA sequences could be "separated by cleavage sequences" as presently claimed. Applicants therefore submit that the specification provides adequate support as to the location of the cleavage sites as presently claimed and that the language "separated by cleavage sequences" does not introduce new matter.

Applicants also note that various site-specific RNA cleavage sites were known in the art at the time of filing. As but several examples: Heck et al., 1999, J. Bacteriol., 181:7621–7625 describes the GAUUUU and GGCUUU cleavage sites for RNAse E of *Rhodobacter capsulans*; Ehretsmann et al., 1992, Genes Dev. 6: 149-159 describes the consensus A/GAUUA/U cleavage site of E. coli RNAse E; Nakamaye & Eckstein, 1994, Biochemistry 33: 1271-1277 describes the cleavage by AUA-cleaving hammerhead ribozyme at AUC, AUA and AUU. Thus, one of skill in the art at the time would have readily seen that Applicants were in possession of the full scope of this aspect of the claims at the time of filing.

Applicants respectfully request reconsideration and withdrawal of this new matter rejection under 35 U.S.C. 112, first paragraph.

2. The Office Action recites the following (page 6, paragraph 2, lines 1-2) with respect to claims 139 and 174:

> "The specification does not contemplate an expression vector wherein the RNA molecule is comprised of different dsRNAs, each being 11-30 nucleotides in length."

Applicants respectfully disagree, for the following reasons.

The specification recites the following (page 9, paragraph 2, lines 1-8):

> "Minimally, to keep the RNA molecule stable, it has a minimum of 11 to 30 nucleotides involved in a double-stranded sequence, depending upon the composition of the polynucleotide sequence and ΔG of about -9.2 kcal/mol. As known in the art, ΔG defines the state of minimal external

> energy required to keep a molecular configuration stable. **Based on this minimum**, preferably at least 10% of this partially double stranded RNA molecule sequence is double stranded."

The specification clearly states that a dsRNA requires at least 11 to 30 nucleotides to be involved in the double stranded sequence. As described above, the invention as presently claimed permits the production of "independently operative dsRNAs," and for such dsRNA to be stable it would be necessary to have at least 11 to 30 nucleotides in the double stranded sequence, as presently claimed. Furthermore, Claims 139 and 174 as previously amended recite "wherein each different double stranded RNA sequence comprises **at least** 11 to 30 nucleotides." One of skill in the art would recognize and understand the need to design each of the "independently operative dsRNAs" generated from the claimed RNA molecule to comprise at least 11 to 30 nucleotides involved in the double stranded sequence for the purposes of stability.

Furthermore, the specification states that a double stranded region of at least 11 to 30 nucleotides is a **minimum** requirement for the characteristics of a dsRNA molecule to be stable. The language "based on this minimum" as recited in the specification further indicates that this requirement applies to any dsRNA described in the specification and that this requirement can be combined with any of the other characteristics for dsRNAs recited in the specification. For example, this minimum requirement can be combined with the description of a multitarget dsRNA on page 20 (paragraph 3, lines 17-24) of the specification to design a multitarget dsRNA that can be cleaved into independently operative dsRNAs, each having a double stranded region of at least 11 to 30 nucleotides. Therefore Applicants submit that the requirement of "at least 11 to 30 nucleotides in a double stranded region" is intended to be a starting point from which a dsRNA with desired properties can be designed, including a multitarget dsRNA that produces independently operative dsRNAs each having 11 to 30 nucleotides in a double stranded region.

Applicants submit that there is adequate description in the specification for designing independently operative dsRNAs that comprise at least 11 to 30 nucleotides of double-stranded character and further submit that the "at least 11 to 30 nucleotides" limitation does not constitute new matter. However, without conceding with regard to the appropriateness of the rejection, Applicants have nonetheless amended claims 139 and 174 to recite “wherein at least 11 to 30

nucleotides of said multitarget partially double-stranded RNA molecule are involved in each different double-stranded sequence." It is believed that the amendment is sufficient to overcome the new matter rejection.

Applicants respectfully request reconsideration and withdrawal of this rejection under 35 U.S.C. 112.

Rejections under 35 U.S.C. § 103

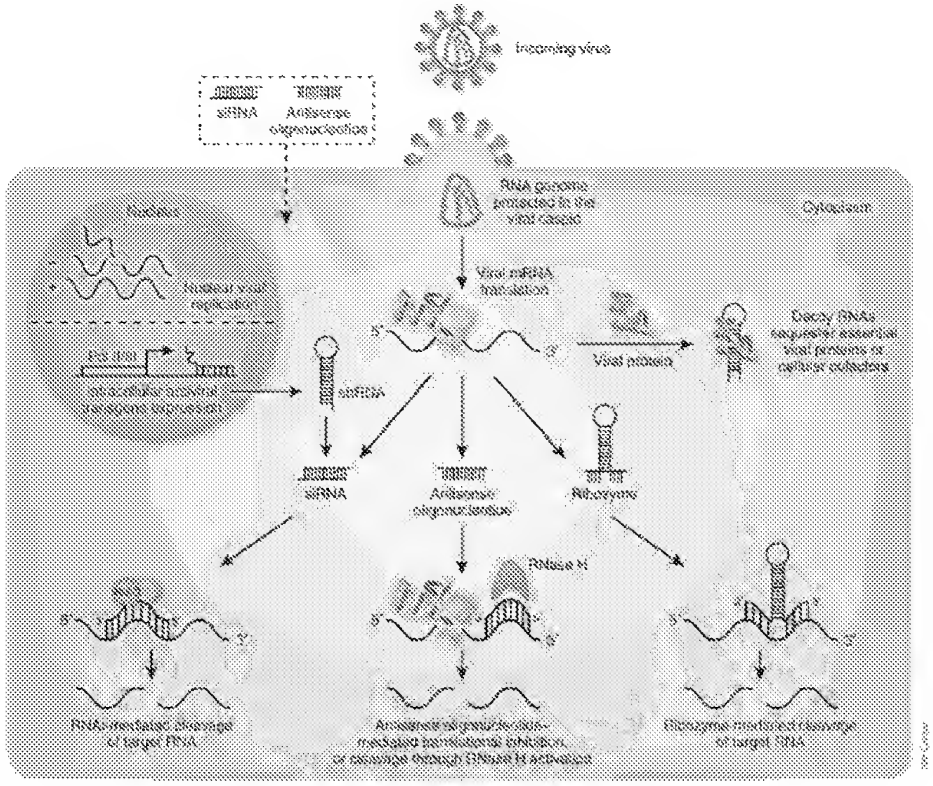
1. Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are rejected under 35 U.S.C. 103(a) based on the combination of Werther et al., Fire et al., Heifetz et al., Calabretta et al., Taira et al., and Thompson et al..

Applicants respectfully disagree with the rejections under 35 U.S.C. § 103(a) for the following reasons.

The Office Action has rejected the claims based on a combination of references that include such diverse expression-inhibiting methodologies as ribozymes, antisense RNA and siRNA. Applicants submit that there is no motivation for one of skill in the art to combine the above-cited references to achieve the invention as presently claimed, because the methods of expression inhibition described in the cited references are not interchangeable.

The invention as presently claimed provides for a partially double-stranded RNA molecule comprising two or more **different double stranded RNA sequences that are substantially homologous and complementary to two or more sequences** of at least one target mammalian gene or mammalian pathogen gene. It is apparent from this description that the *double stranded* regions of the RNA sequences are homologous and complementary to the target gene. Thus, while it is acknowledged by Applicants that the present claims are composition claims, it is clear from the claim language itself, and from the specification that the double-stranded region of the claimed molecules interacts with the target gene RNA sequence. This is in contrast to the mechanism involved in both ribozyme and antisense-mediated expression inhibition, where the *single stranded regions* of the effector RNA molecule permit target recognition. The different processes and mechanisms of action of these expression-inhibiting methodologies, as well as exemplary

structures of the RNA molecule for each method, are shown herein in the following figure from Haasnoot, J et al., (2007) *Nature Biotechnology* 25:1435-1443:



The diagram indicates the regions of each RNA molecule that are necessary for binding to the target mRNA. One of skill in the art would readily recognize that the target recognition regions of an RNA molecule for each expression inhibiting method are very different. In addition, the skilled artisan would recognize that the partially double stranded RNA molecules as presently claimed are directed to an siRNA-like mechanism, since the claim recites "*double stranded sequences* that are substantially homologous and complementary to...at least one target....gene" Further, the skilled artisan would understand that due to these differences in structure and mechanism of action, the considerations (e.g., synthesis, administration, etc.) involved in siRNA-mediated RNA silencing are different from that of both ribozyme and antisense-type expression inhibition.

Taira et al. and Thompson et al. are directed at methods for inhibiting target gene expression by the introduction of ribozymes to a cell. The structure and mechanism of action of a ribozyme for expression inhibition is vastly different from that of a dsRNA of the presently claimed invention. A ribozyme is an RNA molecule with catalytic activity that actively degrades mRNA of a target gene to be downregulated. A ribozyme binds to a target mRNA via its *single-stranded region*, while the dsRNA molecule as presently claimed binds a target mRNA via its *double stranded region*. Thus, due to the different target recognition regions in the molecules of the present invention compared to the target recognition regions in molecules used for ribozyme-mediated inhibition of expression, these methods and the RNA molecules that mediate them are not equivalent and cannot be substituted for one another. One of skill in the art would not be motivated to use the methods of Taira et al., and Thompson et al. in combination with the other cited references, since the processes are different and require different structural considerations, as well as distinct treatment methodologies. The structural differences, especially differences in the target recognition regions of the RNA molecule relative to those of the presently claimed invention are equally true with the antisense molecules as described by Werther et al., and Calabretta et al.

Werther et al., and Calabretta et al. are directed at methods for inhibiting expression by the introduction of antisense molecules to a cell. Antisense molecules are generally *single stranded* RNA molecules that are complementary to a target mRNA molecule. Once introduced into a cell the mRNA and the antisense molecule form a duplex molecule that can be degraded by RNaseH, resulting in inhibition of expression of the target mRNA. The mechanisms and structures required by both ribozymes and antisense molecules are distinct from the invention as presently claimed as, for example, only the claimed invention provides a molecule with a target recognition region present within a double stranded region. One cannot simply substitute structures within one method of expression inhibition for another.

Applicants submit that each of the above-described methods of expression inhibition involve different processes, different molecular structures, and different mechanisms of action to effect inhibition of expression, and are therefore not interchangeable. Despite similar end results, the structures, mechanisms, and methods of ribozyme, antisense, and siRNA mediated inhibition

are not equivalent. Furthermore, it is clear from the claim language that the composition as presently claimed provides for a double stranded RNA molecule that interacts with a target mRNA *via the double stranded region* of the double-stranded RNA and therefore does not represent a ribozyme or antisense RNA molecule. This is further emphasized by the amendment of claim 107 herein to recite "wherein the double stranded RNA sequences mediate inhibition of target gene expression." Thus, the invention as presently claimed is structurally and functionally distinct from ribozyme and antisense-mediated inhibition or inhibiting molecules. Therefore, Applicants submit that one of skill in the art would not have been motivated to combine the teachings of the above-cited references to achieve the invention as presently claimed. Applicants further submit that one of skill in the art would recognize that each method of inhibition requires consideration of unique factors for effecting expression inhibition and one would not be motivated to simply substitute one method of inhibition for another, nor would one be motivated to substitute *parts* of the structure required in one mechanism for parts of the structure required in another mechanism. As such, there would be no motivation to combine the teachings of the cited art as proposed.

Applicants respectfully request reconsideration and withdrawal of the rejections of claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 under 35 U.S.C. 103(a).

2. Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are rejected under 35 U.S.C. 103(a) as being obvious based on the combination of Taira et al., Fire et al., and Thompson et al..

Applicants respectfully disagree, for the following reasons.

As described above in further detail, the Taira et al. and Thompson et al. references are directed at methods of expression inhibition involving ribozyme-mediated cleavage of a target mRNA. Fire et al. is directed to methods of RNA silencing using siRNA-mediated techniques. siRNA are short interfering RNA molecules that bind a target mRNA and promote its degradation by cellular RNA degradation pathways (e.g., RISC formation), while ribozymes actively catalyze cleavage of a target mRNA.

One cannot simply interchange characteristics of a ribozyme with those of an siRNA, since the inhibition processes are different and require different considerations to effectively induce inhibition of expression. For example, the structure of a dsRNA molecule (e.g., siRNA) permits the formation of a RNA-induced silencing complex (RISC), while a ribozyme would not induce such a cellular response due to the differences in molecular structure. Furthermore, the invention as presently claimed is a composition wherein the double stranded region is complementary to the target gene, while both ribozymes and antisense use single stranded regions for binding a target gene (described in more detail above).

Given the clear differences in the structures and mechanisms involved in ribozyme-mediated inhibition, antisense-mediated inhibition and RNA interference-mediated inhibition, Applicants submit that one of skill in the art would not have been motivated to combine the above-cited references to achieve the invention as presently claimed. Applicants further submit that one of skill in the art would recognize that each method of expression inhibition requires consideration of unique factors for effecting expression inhibition and would not be motivated to simply substitute one method of RNA inhibition for another. Applicants submit that the composition as presently claimed is distinct from ribozymes (as described by Taira et al., and Thompson et al.) and antisense molecules (as described by Werther et al., and Calabretta et al.) and that combination of the teachings relating to these methodologies as proposed by the Office Action is not proper. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. § 103(a) rejections based on Taira et al, Fire et al., and Thompson et al.

Conclusion

In view of the amendments to the claims and the reasons discussed above, Applicants respectfully submit that the claims fully comply with 35 U.S.C. 112, and further are non-obvious over the cited references for the reasons discussed above. Applicants respectfully request that the rejections under 35 U.S.C. § 112 and 35 U.S.C. § 103(a) be withdrawn.

In view of the above, all issues raised in the Office Action are addressed herein. Reconsideration of the claims is respectfully requested.

Should any other fees be associated with this submission, the Applicants herewith authorize the Commissioner to charge such fees to Nixon Peabody Deposit Account No. 50-0850. Any overpayments should also be credited to said Deposit Account.

Respectfully submitted,

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